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**14. ABSTRACT**

Breast cancer incidence is highest in caucasian women, lowest in American Indian women, but these trends are reversed for type 2 diabetes. We hypothesized that distinctions in insulin load and signaling may play a role in both diseases and investigated the role of a tyrosine phosphatase, PTP1B, previously reported to be a regulator of both insulin signaling and breast cancer. We noted that calcium flux into breast cancer cells suppressed tyrosine phosphorylation and induced partial proteolysis of PTP1B, resulting in liberation of PTP1B from its membranous anchor. To investigate the role of the cytoplasmic form of PTP1B (tPTP1B) in breast cancer cells, we expressed it and various mutants (phosphatase-dead, substrate-trap) in breast cancer cells under control of an inducible promoter. Unexpectantly,tPTP1B did not suppress insulin signaling but targeted phosphorylated HER2, suppressing its signaling. Our results suggest that activation of PTP1B by its partial proteolysis targets HER2, an oncogene common in aggressive breast cancer and modulation of PTP1B proteolysis through calcium flux may be a unique approach in treatment or prevention of breast cancer.

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INTRODUCTION

Tyrosine phosphorylation is regulated by the controlled activation of protein tyrosine kinases and phosphatases (PTP). While kinase activation occurs primarily through auto- and trans-phosphorylation, phosphatase activation is controlled by a variety of mechanisms. One example of distinct phosphatase regulation is PTP1B, a highly expressed tyrosine phosphatase that exists as an endoplasmic reticulum (ER)-associated protein (1-3). The hydrophobic C-terminal domain of PTP1B directs its association with the outer leaflet of the ER membrane such that its catalytic activity extends into the cytosolic compartment (4-7). Importantly, PTP1B has been shown to dephosphorylate several proteins including those that regulate hormone (insulin), growth factor (EGF, IGF) and cytokine (prolactin, leptin) activity (8-14). Therefore regulation of PTP1B activity may play an important role in tumors dependent of these signaling entities or in metabolic disorders such as diabetes. Previous studies demonstrated that PTP1B activity was controlled by its expression, phosphorylation and limited proteolysis (4-7). Activation of the Ca+2 sensitive protease calpain by increased cytosolic calcium levels results in limited proteolysis of PTP1B through cleavage of its hydrophobic C-terminal tail, releasing PTP1B enzymatic activity into the cytosol where signaling intermediates can be utilized as substrates and undergo dephosphorylation (4-7,15). Limited PTP1B proteolysis may provide a novel mechanism for phosphatase activation and regulation of growth factor and cytokine signaling (figure 1) (5,7,15,16). To test this hypothesis, a C-terminal truncated form of PTP1B (tPTP1B; ER-localization domain deleted) was expressed in breast cancer cells and its effects on growth, apoptosis, and target protein dephosphorylation were examined. We used transient transfection and an inducible expression system to evaluate the changes associated with expression of tPTP1B and several mutants that either possess no phosphatase activity (C215S) or trap phosphotyrosyl-protein substrates (D181A) (17,18). Induction of tPTP1B, but not tPTP1B/C215S, suppressed the growth of breast cancer cells (MCF-7, SKBr-3). tPTP1B did not reduce insulin-mediated signaling (Akt activation) but suppressed phosphorylation of Her2 in cells with either high or low levels of Her2. Further, tPTP1B suppressed Jak2 activation and signaling. Overall, our results provide evidence that truncation and cytosolic localization of PTP1B results in suppression of specific tyrosine

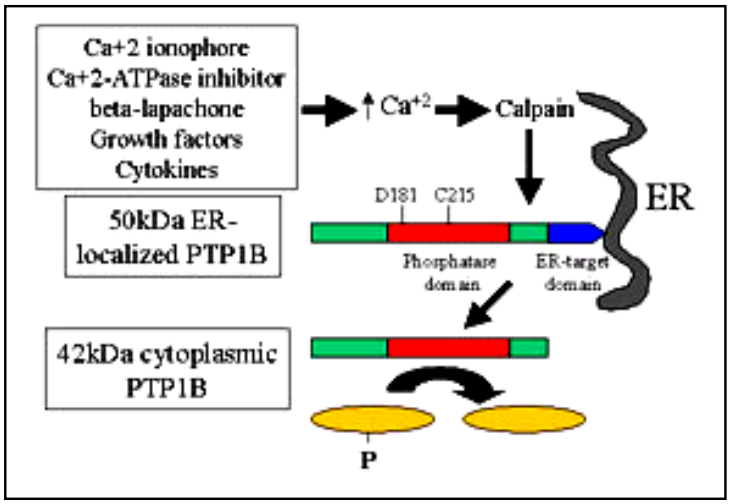


Figure 1. Proposed mode of PTP1B cytosolic translocation. The phosphatase (red) and ER-localization domain (blue) as well as two mutants (D181A, C215S) used in these studies are also depicted.

kinases expressed in breast cancer cells that mediate gene expression and transformation. Since PTP1B can undergo limited proteolysis in response to increased intracellular calcium flux (possibly through activation of calpains) it may suppress tumorigenicity by increasing cytosolic PTP1B levels and suppression of Her2 and Jak2 phosphorylation. Compounds that increase cellular calcium levels in breast tissue may potentiate PTP1B truncation and suppress the onset of Her2-dependent and cytokine-responsive breast tumors.

BODY

Two specific aims and a total of 9 tasks were proposed to complete this study. We have addressed both specific aims and completed most of the tasks assigned to each aim. The important and salient discovery was that expression of tPTP1B reduced growth of breast cancer cells and targeted HER2 and Jak2 kinases. We did not detect evidence for apoptotic induction through expression of tPTP1B alone.

Specific aim 1. To examine the effect of cytoplasmic PTP1B expression on apoptotic sensitivity of breast carcinoma cells.

Four tasks were assigned to accomplish this specific aim. These include the completed subcloning of PTP1B into the pcDNA3 vector with an engineered FLAG tag for simple western identification. Cells were to be transiently transfected and monitored by western blot. Optimal expression conditions were to be assessed. Apoptotic sensitivity was also to be tested by cotransfection with a reporter construct (galatosidase). These experiments were to be performed in MCF-7 and SKBr-3 cells.

Most of these tasks were completed and reported in the previous year but several modifications were made due to the limitations of this particular expression system. We initially demonstrated pCMV-Flag tagged tPTP1B (PTP1B/400) in MCF-7 cells and showed that coexpression with a reporter gene did not increase apoptotic sensitivity (compared to empty vector alone) and apoptotic sensitization did not appear to be dependent on PTP1B phosphatase activity since phosphatase-dead PTP1B mutants had similar affects. This activity was unexpected and needed to be confirmed by alternate approaches. Therefore, we subcloned the cytoplasmic form of PTP1B (tPTP1B) into additional expression vectors to obtain stable transfectants or inducible expression of the phosphatase active protein and its phosphatase-dead or substrate-trapping mutants.

We were never able to obtain stable transfectants using the pCMV expression plasmid. Therefore, we analyzed cellular responses to transient transfection using several a FLAG-tagged PTP400 (pCMV-3X-Flag-10; Sigma), a GFP-retroviral expression vector (pLEGFP-C1; Clontech), three tet repressor expression constructs for tet-regulated PTP400 expression (pEC1214A; in-house construct provided by Dr. Xu (19), Dept. of Molecular Oncology), cDNA4/TO and pcDNA4/TO/myc-His; Invitrogen). We have subjected cells to G418 selection and recovered stable transfectants.

First, we demonstrate that transfection with the pLEGFP-C1/tPTP1B vector resulted in recovery of tPTP1B expressed throughout the cytoplasm (figure 2) and it was associated with reduced phosphorylation of several tyrosyl phosphoproteins (figure 2). These initial studies suggested that HER2 was a potential target for tPTP1B since

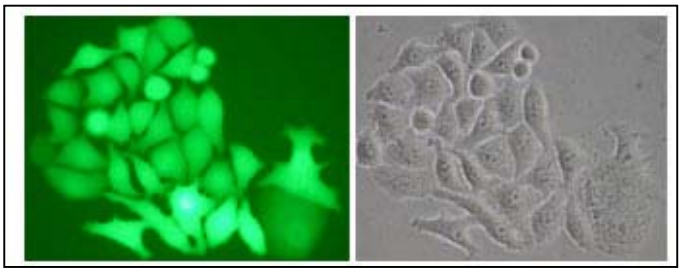

Figure 2. Detection of cellular GFP-tPTP1B levels in transfected MCF-7 cells. Left – GFP-tPTP1B fluorescence, Right – Phase contrast microscopy.

immunoprecipitation of substrate trapping versions of tPTP1B expressed in SKBr-3 resulted in recovery of HER2. However, assessment of long-term stable transfectants suggested that tPTP1B-eGFP expression was diminished with time and was independent of PTPase activity and after 1 month of G418 selection we obtained G418 resistant cells but only a limited number retained eGFP-tPTP1B expression. Therefore, additional inducible expression systems were explored.

We initially sub-cloned the eGFP-tPTP1B insert into the pEC1214A vector provided by Dr. Xu. This vector was attractive since it allowed constitutive expression of the tetracycline repressor, a cloning site that allows insertion of a gene (tPTP1B) that is controlled by the Tet repressor and the neomycin resistance gene. MCF-7 cells were transfected with the pEC1214A (pTet), and pTet-tPTP1B vector using Lipofectamine as described by the manufacturer (Life Technologies). A stable pool of MCF-7 cells harboring either pTet or pTet-tPTP1B selected by growing the cells in complete medium containing tetracycline (1 μg/ml) and G418 (600 μg/ml). Induction of tPTP1B occurred after the withdrawal of tetracycline. However the level of tPTP1B protein expression induced upon tetracycline withdrawal was not sufficient for assessing function as it did not approach the level achievable with partial proteolysis of PTP1B in A23187 treated cells (data not shown). Again, additional inducible expression systems were tested.

We have used a tetracycline-inducible T-REx expression system which consists of two key expression vectors pcDNA4/TO and pcDNA6/TR (20). The pcDNA4/TO vector encodes two tetracycline operator sites (tetO) for tetO-regulated gene expression in mammalian cells. In the presence of the tetracycline repressor (TetR) that is expressed by pcDNA6/TR, the tetO sites in pcDNA4/TO-E are bound with TetR and transcription is repressed. In the presence of tetracycline, tetracycline binds to TetR and releases TetR from the tetO sites, allowing transcription to proceed. This allows increased tetracycline-inducible expression of target genes in a cell line of interest.

To allow tetracycline-inducible expression of a N-terminal eGFP-tPTP1B fusion protein and mutant constructs (D181A and C215S) gene inserts were restricted out of the pLEGFP-C1 vector and sub-cloned into the Bam H1 and Xba 1 sites of the pcDNA4/TO vector from Invitrogen's T-Rex system using gene specific primers. A cell-line optimization nucleofector kit was purchased from Amaxa Biosystems to optimize the electroporation of both the MCF7 and SKBr3 cell lines and used according to the manufacturer's protocol. We determined that 70 % transfection efficiency was achieved by using 2 million cells in solution V for both cell lines. Setting Q-27 was used for MCF7 and setting A-23 for SKBr3. Initially cells were electroporated with pcDNA6/TR and selected for stable expression of TetR using 5 μg/ml blasticidin. Individual foci were selected and expanded and tested for tetracycline-inducible gene expression by transient transfection with eGFP in the pcDNA4/TO vector. MCF-7 and SKBr-3 clones that

demonstrated highest eGFP expression determined by FACS scanning (BD FACS Vantage SE) in the presence (vs. absence) of 1 μg/ml tetracycline) were selected for subsequent electroporation of eGFP-tPTP1B and its point mutants cloned into the pcDNA4/TO vector. Transfected cells were selected for zeocin (200 μg/ml) and blasticidin (5 μg/ml) resistance to obtain stable cell lines with inducible tPTP1B expression. Twenty-four hours after tetracycline addition, cell lysates were assessed for eGFP positivity (by FACS) and eGFP-tPTP1B expression by immunoblotting. As shown in figure 3, although some tPTP1B expression was evident in the absence of tetracycline, this tet inducible expression system gave adequate inducible expression of tPTP1B to allow assessment of its role in breast cancer cell growth and signaling.

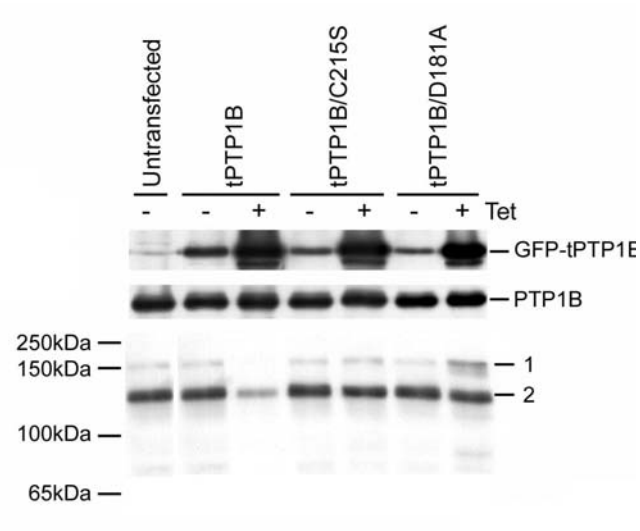


Figure 3. Tet inducible expression of tPTP1B in transfected MCF-7 cells. Two phosphoproteins (#1, ~170kDa and #2, ~125kDa) were affected by tPTP1B induced expression.

To complete aim 1, equal numbers of MCF-7 and SKBR-3 cells expressing tet-inducible tPTP1B were cultured in the presence of tetracycline and cell density was analyzed after 4 days. Trypan blue exclusion was also evaluated to determine whether tPTP1B expression effected cell survival. As shown in figure 4, both SKBr-3 and MCF-7 cell growth was significantly suppressed (* $p < 0.05$) in tetracycline-treated cells expressing phosphatase-active tPTP1B. Neither basal nor tet-inducible phosphatase-dead (C215S) or substrate-trap mutant (D181A) forms of tPTP1B significantly suppressed MCF-7 or SKBr-3 cell growth. Importantly, trypan blue exclusion was not effected by tPTP1B induction, suggesting that the affect of this phosphatase on breast cancer cells were primarily mediated by cell cycle restriction. Additional cell cycle analysis is required to confirm this mechanism. Overall, the results thus far suggest that tPTP1B is active in reducing

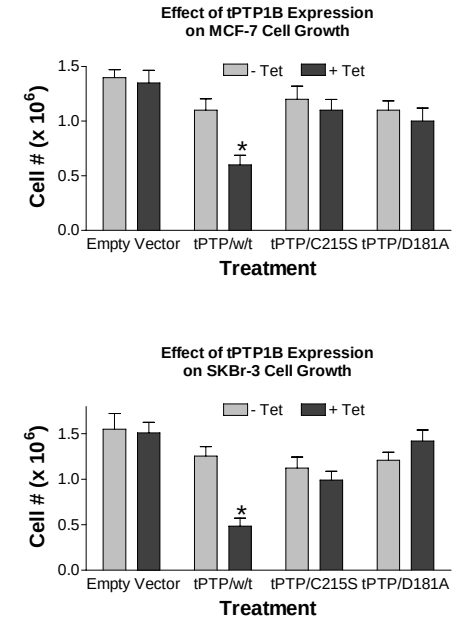


Figure 4. Effect of Tet-induced tPTP1B expression on breast cancer cell growth.

breast cancer cell growth without induction of apoptosis and was active in cells expressing high (SKBr-3) and low (MCF-7) levels of the HER2 oncogene. From earlier studies we determined that partial proteolysis of PTP1B through calcium-mediated activation of calpain resulted in an increase of tPTP1B in the cytoplasmic fraction (4-8). The results presented here demonstrate that this form of PTP1B is active in suppressing breast cancer cell growth through a tyrosine-phosphatase dependent mechanism. These results also support evidence of a unique intracellular calcium responsive cascade involving calpain and PTP1B in the suppression of breast cancer growth. Agents that increase intracellular calcium levels may suppress breast cancer growth through this mechanism.

Specific aim 2. To examine whether expression cytoplasmic PTP1B alters insulin (or IGF-1) signal transduction and survival pathways in breast carcinoma and whether HER2 expression alters PTP1B-mediated apoptotic sensitization.

To complete these studies 5 tasks were proposed. We initially subcloned the tPTP w/t and mutant variants (D181A, C215S) into the tet-repressive vector pEC1214A (as reported above). Breast cancer cell lines were transfected with FUGENE and 2 μg of DNA and cultured in the presence of tetracycline (to modulate tPTP expression). Tetracycline (tet) was removed and tPTP expression was assessed by immunoblotting after 48 hours. Cells expressed tPTP in the absence of but not presence of tet were further selected in the presence of G418 (neomycin). At least 5 stable clones from each transfectant were analyzed for tPTP in the absence and presence of tet. Unfortunately stable integration of the pEC1214A vector resulted in minimal control of tPTP expression and these clones were of little use for these experiments. Therefore, additional vectors (pEGFP-C1 and the 2 plasmid T-Rex system, from Clontech and Invitrogen, respectively; described in specific aim 1) were tested for their ability to provide efficient and inducible expression of tPTP (21). Task 1 and 2 of this aim were completed.

To address tasks 3 and 4, we used an electroporation system for transient transfection that results in 60 to 80% transfection efficiency (AMAXA Technology). We also employed standard transfection methodology but sorted GFP positive cells before analysis.

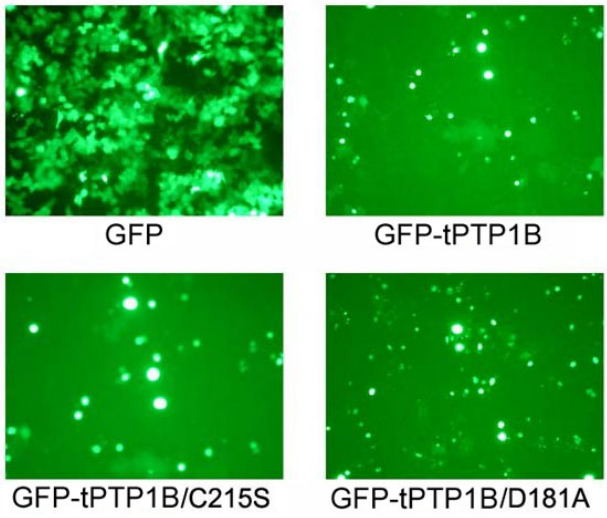

Figure 5. Recovery of GFP-tPTP1B expressing MCF-7 cells after FACSorting.

To address task 3, tPTP w/t and 2 mutants (D181A, C215S) were subcloned into the pEGFP-C1 vector so that tPTP1B was expressed as a fusion protein composed of eGFP and tPTP1B so that tPTP1B was expressed as a GFP fusion protein with GFP at the N-terminus (21). MCF-7 cells were transfected ($10^6$ cells) with 5-10 μg of DNA (FUGENE) and after 48 hours GFP (+) cells were sorted by

FACS in our institutional core facility (MD Anderson Cancer Center). One hundred thousand cells were obtained for each construct and these cells ere placed in culture overnight before analysis of GFP-tPTP expression by fluorescent microscopy and immunoblotting which demonstrated cytoplasmic localization and expression of GFP-tPTP (figure 5).

Cells were maintained in culture for 7 days and we noted that GFP-tPTP (+) cells were largely detached from the culture dish. GFP expression alone did not alter cell adhesion. Detached cells were removed and examined for markers of apoptosis, but caspase cleavage and trypan blue exclusion analysis failed to support evidence of cell death. Remaining cells were subjected to fluorescent microscopy. These experiments suggested that this tPTP-expression vector induces changes in cell viability that is independent of phosphatase activity. Recent reports suggest the multiple cloning sites in the pEGFP vector (not EGFP expression itself) underlie the cytotoxicity of this vector (22). These results were not anticipated and may explain the difficulty associated with establishment of stable tPTP expressing cells using this expression vector. However, transient transfection with this vector was used initially to screen for the impact of tPTP1B expression on control and growth factor-activated signaling.

To address task 4 and 5 we monitored the effects of growth factors (insulin, heregulin, EGF) on tyrosine phosphorylation. As shown in figure 6, insulin, EGF and heregulin increased tyrosine phosphorylation in MCF-7 cells but differed in their pattern of phosphotyrosine induction. We were most interested in the effects of insulin on signaling proteins since previous studies suggested PTP1B interferes with this cascade.

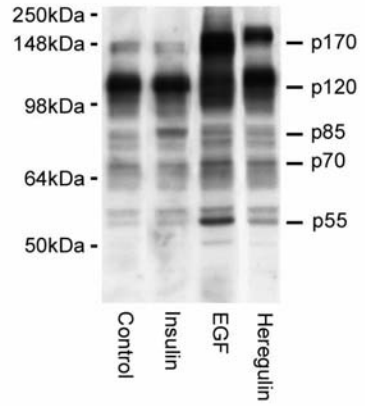


Figure 6. Treatment induced changes in tyrosine phosphoproteins in MCF-7 cells.

The effects of GFP-PTP construct expression on total and insulin-stimulated tyrosine phosphorylation were examined in MCF-7 cells. MCF-7 cells were transfected with various EGFP-tPTP constructs or EGFP vector alone using FUGENE. After 72 hours, transfectants were flow sorted for EGFP positivity and the EGFP-enriched population was placed in culture media overnight before treating the cells with insulin or diluent as a control. As shown in figure 7, EGFP-tPTP1B w/t and mutant expression was detected and only minor changes in total tyrosine phosphorylation were noted (labeled #1 -5). However, three interesting observations were made. First, insulin stimulation resulted in high level activation of Akt phosphorylation (S473), which is known to be a critical downstream component of the insulin/PI3'kinase cascade (23,24). High level expression of tPTP1B did not effect this activation, suggesting that tPTP1B is distinct from the ER-localized PTP1B molecule which was previously reported to block insulin signal transduction (9-11). Second, src activation was increased in tPTP1B w/t expressing cells in agreement with earlier reports of ER-localized PTP1B mediated c-src activation in breast cancer (25,26). Src activation is though to be mediated by dephosphorylation of the negative regulatory Y530 site (25). Therefore tPTP1B and PTP1B share some, but not

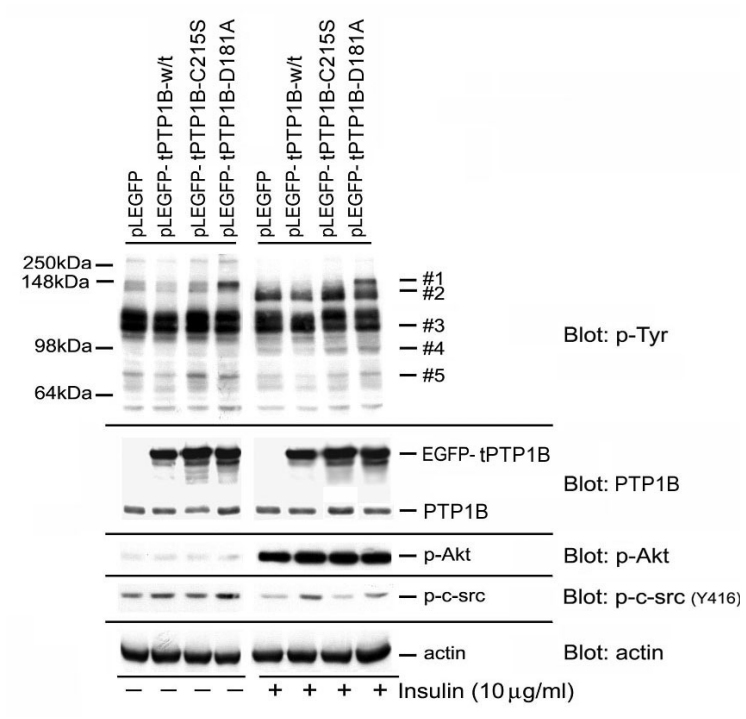


Figure 7. Effect of EGFP-t-PTP1B on tyrosine phosphorylation, insulin-mediated Akt and c-src phosphorylation in MCF-7 cells.

all previously reported substrates and activities. Lastly, a unique phosphotyrosyl protein was detected in cells expressing the substrate-trap mutant of tPTP1B. Substrate-trap PTP1B is able to bind and stabilize the tyrosine phosphorylation of its high affinity substrates (17,18). Expression of this pY (>148kDa) was present in control and treated cells and its basal phosphorylation was not effected by insulin. The identity of this protein was a key focus of subsequent experimentation.

To further address the nature of the pY protein detected in tPTP1B-D181A expressing cells, parallel studies of the effect of tPTP1B expression in MCF-7 and Her2 overexpressing SKBr-3 breast cancer cells were conducted. Expression of the w/t tPTP1B resulted in complete loss of Her2 tyrosine phosphorylation in SKBr-3 cells (figure 8). Phosphatase-dead tPTP1B (C215S) had no effect on Her2 phosphorylation while increase tyrosine phosphorylation was detected in cells expressing the substrate trap tPTP1B mutant. None of the tPTP1B constructs affected Her2 protein levels. These results suggest that Her2 was a potential substrate of tPTP1B.

To confirm this hypothesis, additional studies were conducted in transiently transfected MCF-7 and SKBr-3 cells. As shown in figure 9, GFP-tPTP1B was expressed in MCF-7 and SKBr-3 cells and 72 hours after

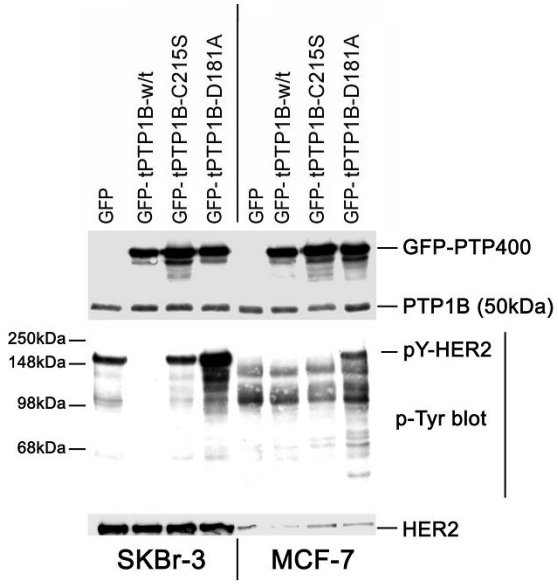


Figure 8. Effect of EGFP-tPTP1B expression on Her2 protein and phosphorylation in breast cancer cells.

transfection, cells were flow sorted for GFP positivity. Cell lysates were prepared and subjected to GFP immunoprecipitation. Lysate from untransfected cells were also subjected to GFP immunoprecipitation and served as a control (lane 1).

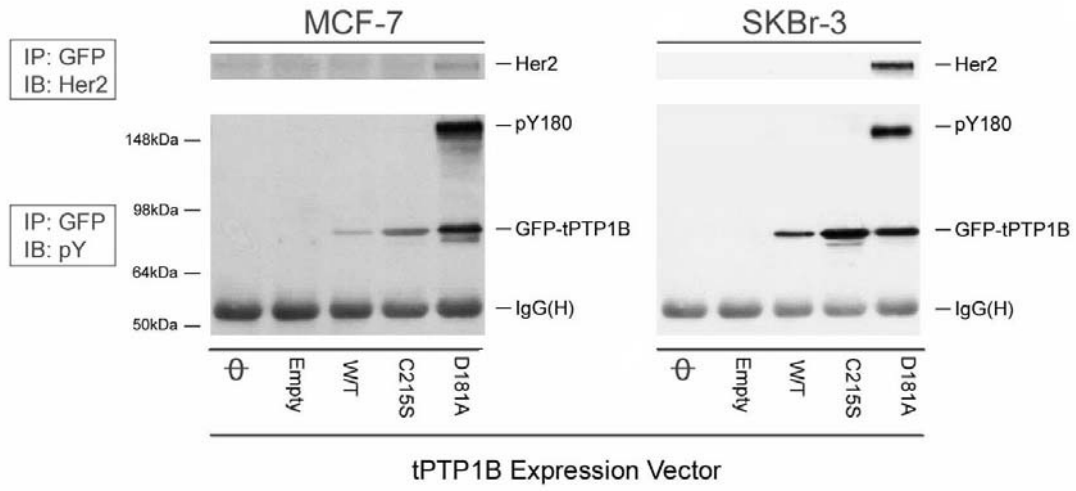


Figure 9. Immunoprecipitation of substrate trapping GFP-tPTP1B/D181A pulls down tyrosine phosphorylated Her2 in breast cancer cells.

Immunoprecipitates were washed 3 times and resolved by SDS-PAGE and the membrane was immunoblotted for pY (bottom) and Her2 (top). Initial pY blots detected GFP-tPTP1B (perhaps due to its tyrosine phosphorylation) and an additional protein >148kDa was detected in lanes expressing the tPTP1B substrate trap. Subsequent blotting for Her2 supported the possibility that tPTP1B recognized tyrosine phosphorylated Her2 as a substrate. Since previous work had demonstrated that overexpression of PTP1B suppressed transformation by Her2 but through an unknown mechanism (27,28), our results suggest that expression of a cytoplasmic form of PTP1B may target Her2 and prevent its full activation, suppressing its transforming activity. PTP1B overexpression may result in its ER and cytoplasmic accumulation or overexpression may result in partial proteolysis of its ER-localization domain (29). Interestingly, substrate-trapping PTP1B expression in fibroblasts recovered EGFR, but not Her2 (17,18). This may due to limited pY-Her2 expression in fibroblasts, or tPTP1B and PTP1B may differ in their preferred substrates. There is also the possibility that GFP-tPTP1B has phosphoprotein target specificities that are distinct from those of PTP1B and tPTP1B ( 15,16).

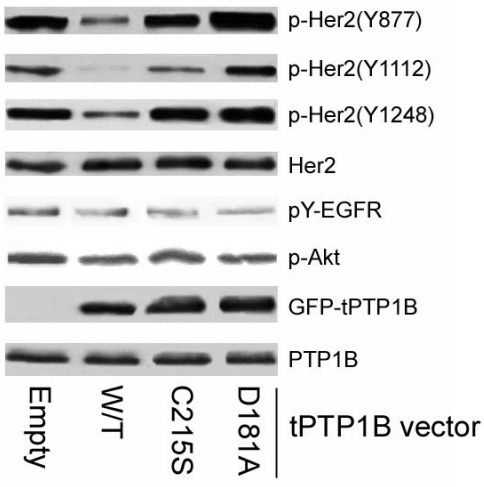


Figure 10. Effect of tPTP1B expression in SKBr-3 cells on Her2 site-specific tyrosine Phosphorylation.

To further assess Her2 targeting by the tPTP1B GFP-fusion protein, tPTP1B constructs were again expressed in SKBr-3 cells and sorted for GFP

positivity (72 h after transfection). Cell lysates were resolved and probed with antibodies against specific phosphosites (Y877, Y1112, Y1248) on Her2, Her2 and other signaling molecules. As shown in figure 10, w/t tPTP1B expression reduced tyrosine phosphorylation at every site examined but did not affect pY-EGFR or Her2 protein levels. Although the expression levels of every tPTP1B construct were similar, the C215S variant did not affect pY-Her2 levels while the D181A mutant partially protected Her2 tyrosine phosphorylation. None of the tPTP1B constructs affect activation of the PI3K/Akt cascade (monitored by pS473-Akt immunoblotting), supporting earlier observations in insulin-treated cells (figure 7). These results confirm that tPTP1B targets Her2 and induces its dephosphorylation.

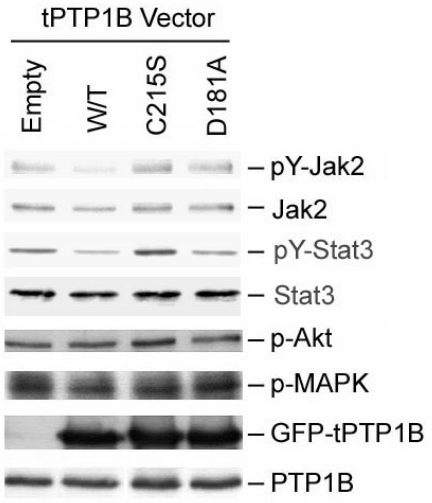


Figure 11. Effect of tPTP1B expression in MCF-7 cell Jak2 and Stat3 activation.

Other kinases previously described as PTP1B substrates were also screened in MCF-7 transfectants. Jak2 has been described as a substrate for PTP1B (12,13) and its endogenous phosphorylation was compared in GFP and GFP-t-PTP1B expressing transient transfectants after flow sorting. As shown in figure 11, w/t tPTP1B suppressed phosphorylation of Jak2, Stat3 and MAPK but did not affect p-Akt. C215S mutant expression had minimal effect on proteins, suggesting that phosphatase activity was required for suppression of target substrate phosphorylation. However, the substrate-trapping mutant did not provide protection from dephosphorylation, suggesting that Jak2 is low affinity substrate or an indirect target of tPTP1B.

To determine whether tPTP1B also affects growth factor signaling the TRex tet-inducible tPTP1B expression system was used. MCF-7 cells stably transfected with the

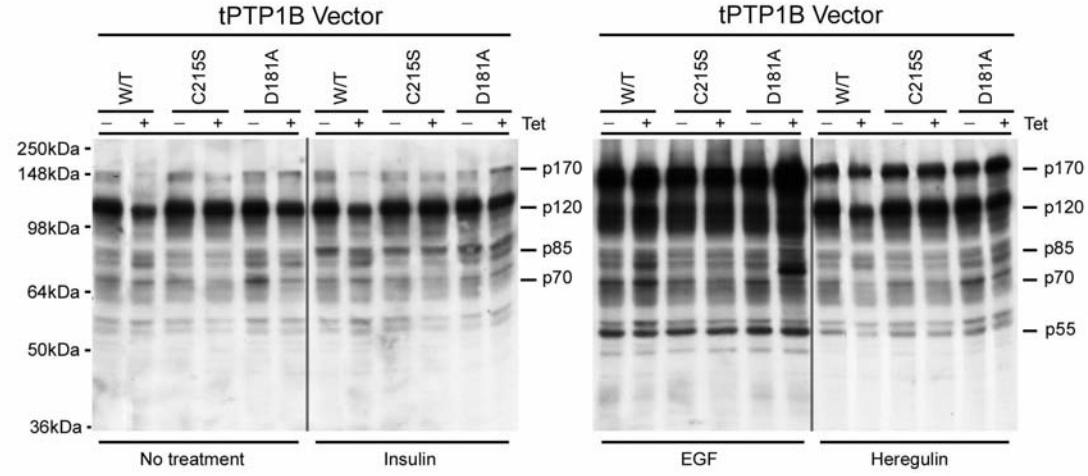


Figure 12. Effect of tet-inducible tPTP1B expression on growth factor signaling in MCF-7 cells.

tPTP1B expression vectors were left untreated or treated with doxycycline (tetracycline) for 48 hours to induce tPTP1B expression. Cells were then left untreated (no treatment, figure 12) or treated with 10 μg/ ml insulin, 100 nM EGF or 100 nM heregulin for 30 min. Cell lysates were collected and phosphotyrosine levels were determined by immunoblotting. In the presence of doxycycline, wt tPTP1B suppressed phosphorylation of both a major (p120) and minor (p170) protein in both treated and untreated cells. Again expression of the C215S mutant had no phosphoregulatory activity in the presence or absence of tetracycline. Interestingly, in the presence of tet D181A expression, the p170 protein band was increased in density, suggesting that the D181A substrate-trapping variant blocked dephosphorylation of the p170 protein. Protected by D181A was enhanced by EGF and heregulin treatment, supporting the previous observations of a role for tPTP1B in regulation of the Her2 phosphorylation.

Overall, the results suggest that tPTP1B recognizes Her2 as a substrate, reducing its level of activation. tPTP1B has little direct effect on insulin signaling. The results suggest that Ca+2 flux into breast cancer cells activates calpain, resulting in partial proteolysis of PTP1B and cytoplasmic accumulation of tPTP1B. In this compartment, tPTP1B interacts with Her2, reducing its level of activation. Calcium accumulation in breast cancer cells may suppress the oncogenicity of Her2 through liberation of tPTP1B into the cytoplasm of these cells. Agents that increase intracellular calcium levels may be effective in preventing the onset of Her2-mediated transformation and Her2 positive breast cancer. More research is obviously needed to support this hypothesis.

KEY RESEARCH ACCOMPLISHMENTS:

1. Demonstrate that calcium flux into breast cancer cells activates calpain, resulting in partial proteolysis of PTP1B and release of this phosphatase from its ER-anchored position. Partial proteolysis results in loss of the ER-localization moiety on PTP1B, resulting in the cytoplasmic accumulation of a truncated form of PTP1B (tPTP1B).
2. The activity of tPTP1B was examined in breast cancer cells and it demonstrated unique substrate interaction and utilization when compared to that reported for PTP1B.
3. tPTP1B interacts with Her2 and reduces its tyrosine phosphorylation. We could not detect site-specific preferences for tPTP1B among multiple sites of Her2 phosphorylation.
4. Insulin signaling (Akt phosphorylation) was not affected by tPTP1B expression.
5. tPTP1B reduced the growth of breast cancer cells.
6. Calcium may suppress Her-2 positive breast cancer cell growth through the actions of tPTP1B.

REPORTABLE OUTCOMES:

This research has matured to the point of publication. The results have been presented at internal seminars at MD Anderson Cancer Center and have generated considerable interest. We will publish the results and provide cell lines to other investigators interested in signal transduction once the tPTP1B vs GFP-tPTP1B comparison is complete. The concern is that the GFP portion of the chimeric gene alters

the distribution of tPTP1B. The project has become more interesting as others are reporting a role for the calpain/PTP1B axis in other signaling pathways.
Part of the research conducted under this grant has been useful in obtaining support for prostate cancer research.

Since initial submission of this research project, substantial new information has accrued regarding substrates for PTP1B (7,15,16,21) and the role of PTP1B in Her2 tumorigenicity (27,28). Jak2 and Stat3 phosphorylation were diminished by tPTP1B but these affects appear to be indirect as there was no "protection" by expression of the substrate trap tPTP1B mutant. Animal models suggest that Her2-mediated transformation of mammary epithelium was enhanced by PTP1B expression (27). However, our results suggest that there are distinctions in the action of PTP1B in Her2-induced transformation and tPTP1B activity in established breast cancer cells. Distinctions may be due to the localization of the phosphatase activity and substrate availability. More through analysis of the affects of PTP1B and tPTP1B in Her2 signaling are necessary to clarify the prospective role of this phosphatase activity in breast cancer.

A graduate student (Hannah Wingate) interested in breast cancer signal transduction rotated through the lab and conducted some of the research described in this report. Dr. Ji Wu (Research Fellow involved in this project) was also recently promoted due, in part, to the research project supported by this grant. A technician (Jonathan Stapley) conducted and designed some of the experiments described in this report. He has subsequently left the laboratory and pursued a business degree with the goal of joining a biotechnology company.

CONCLUSION:

The results suggest that Ca+2 flux into breast cancer cells activates calpain, resulting in partial proteolysis of PTP1B and cytoplasmic accumulation of tPTP1B. In this compartment, tPTP1B interacts with Her2, reducing its level of activation. tPTP1B has little direct effect on insulin signaling. Calcium accumulation in breast cancer cells may suppress the oncogenicity of Her2 through liberation of tPTP1B into the cytoplasm of these cells. Agents that increase intracellular calcium levels may be effective in preventing the onset of Her2-mediated transformation and Her2 positive breast cancer. More research is obviously needed to support this hypothesis.

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APPENDICES:

None